Selecting Increased Seed Density to Increase Indirectly Soybean Seed Protein Concentration

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ABSTRACT

Because soybean [*Glycine max* (L.) Merr.] is the world's most important source of high quality vegetable protein, development of high yielding genotypes with increased seed protein concentration is a major soybean breeding objective. A major impediment to this objective is the often observed negative correlation between yield and protein. Seed density is a component of grain yield that is correlated positively with seed protein concentration. If genotypic correlations between seed density and yield are low, selection for increased density could provide an efficient way to improve protein concentration without affecting seed yield. The objective of this study was to investigate direct and correlated responses to selection on density of seeds sampled from male-sterile plants in three different random-mating populations. Seed density was determined for 192 male-sterile plants in each population. In each population, 15 plants with the highest and 15 plants with lowest seed density were selected. Seeds of each selection were increased in the winter and tested the following summer at three locations in North Carolina with two replications per location. In those tests, the previously selected high and low density groups were not significantly different in seed density, seed weight, yield, or concentrations of protein and oil. Thus, single plant selection for seed density was ineffective for increasing seed density or seed protein concentration. An alternative selection method is proposed in which the selection unit is a selfed half-sib or S_1 family. Desired gains selection indices for increased density and seed weight may increase both protein and yield in all three populations. This selection system has appeal because measurement of seed density and seed weight is relatively inexpensive, requiring less economic and land resources than actual measurement of yield and protein. It is recommended as a low-cost way to improve initially unadapted populations.

Soybean is the most important source of edible vegetable oil and high quality vegetable protein in the world. It supplies about one-fourth of the world's edible oils and two-thirds of the world's protein meal production (Golbitz, 2001). Soybean protein has an excellent balance of amino acids compared with other vegetable proteins (Wolf and Cowan, 1975). Thus, development of high yielding soybean genotypes with increased seed protein concentration is desirable and has become a major objective of some soybean breeding programs.

Soybean seed protein concentration is a trait with relatively high heritability. In six separate studies involving 13 populations of random lines from two-way crosses, mean heritability in populations from crosses between adapted lines with average protein concentration was 0.71, and the mean heritability in populations from crosses where one or more of the parents had above average protein concentration was 0.82 (Burton, 1985). By comparison, average heritability for seed yield was 0.37 in studies of eight populations (Burton, 1987). While efforts to increase both traits individually have been successful, increases in both traits simultaneously have been rare. Estimates of genetic correlation between yield and protein in breeding populations are usually negative (Burton, 1985, 1987). It is sometimes possible to increase yield or protein without decreasing the other. Brim and Burton (1979) increased protein concentration in one population through six cycles of recurrent S1 family selection without reducing seed yield. Holbrook et al. (1989) successfully used restricted index selection to increase yield while maintaining protein concentration at a moderately high (455 g kg^{-1}) level. Wehrmann et al. (1987) were able to increase protein concentration with two backcrosses and recover the yielding ability of the recurrent parent. Wilcox and Cavins (1995) have been the most successful using backcross methodology, with selection of a line in the BC_3 generation that was higher yielding than the recurrent parent, 'Cutler 71', and 64 g kg^{-1} higher in seed protein concentration.

All of the previously cited selection methods require two or more years per cycle. Recurrent mass selection could be a more rapid alternative method. Tinius et al. (1991) were able to increase yield indirectly by practicing mass selection for seed size in three replicate soybean populations. In one of the replicates, yield increased without a decrease in protein (Tinius et al., 1993). Soybean populations described in the above studies were segregating for the ms_1 male sterile (MS) gene, and mass selection was imposed on the seed phenotype of MS plants. The existence of male sterility allowed insect-mediated random intermating of selections in each cycle and facilitated the completion of a cycle of selection each year with the use of a winter nursery to increase the seed of selected individuals. In the population where yield increased and protein remained constant, seed density increased (M.H. Yang and J.W. Burton, unpublished data, 1992). Because seed density has been used to evaluate seeds for protein content (Hartwig and Collins, 1962), it could potentially be used for indirect selection of protein content.

Seed density is a component of grain yield. That is, seed yield (weight per unit area) is the product of seed number (number per unit area), seed density (weight per mm^3), and volume (mm^3 per seed). The heritability of seed density is generally higher than that of seed yield but lower than that of seed weight. Johnson and Bernard (1963) reported the average heritability of seed density from several studies to be 0.47. Fehr and Weber (1968) obtained estimates of heritability for seed density from 0.83 to 0.88 in two populations. Estimates of geno-

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Abbreviations: MS, male-sterile; MF, male-fertile.

typic correlation between seed density and protein range from 0.06 to 0.71 (Fehr and Weber, 1968; Smith and Weber, 1968). Also, a regression of seed density on protein concentration of 41 standard soybean calibration samples was linear (Li, 1996). Generally, seed density has been found to be weakly related to seed yield with genotypic correlations between −0.20 and 0.41 (Fehr and Weber, 1968; Smith and Weber, 1968). Thus, selection on seed density could increase protein concentration without decreasing seed yield. The objective of this study was to investigate direct and correlated responses to selection on density of seeds sampled from MS plants in three different populations.

MATERIALS AND METHODS

The three populations in this study were designated Population II, Population III, and Population VII. Population II was the intermating population N70-1400 (Burton and Brim, 1981). N79-1400 was synthesized by mating the 10 highest-yielding lines from a selected population, YC2 (Kenworthy and Brim, 1979), to an ms_1 male-sterile maintainer line, N69-2774 (Brim and Young, 1972). Population III was the intermating population N79-1500, which was formed by mating N69-2774 to six high-yielding cultivars or breeding lines that were highly adapted to the Southern USA (Burton and Brim, 1981). Both N79-1400 and N79-1500 were released after eight generations of random intermating without selection. Prior to initiation of this experiment, both populations had undergone 13 additional generations of random mating. Population VII was formed from a randomly intermated population derived by crossing the fourth cycle of selection from a high oil recurrent selection population (Burton and Brim, 1981) with the fourth cycle of selection from a high protein recurrent selection population RS 4 (Holbrook et al., 1989). The high oil population was segregating for ms_1 male sterility, so intermating was done by insect mediated pollination. To accomplish this, two populations were interplanted by placing one seed from each in single hills. At maturity, only seeds from male sterile plants were harvested. Prior to the initiation of this experiment, there were five generations of random intermating without selection in this population.

Experimental Procedure

Identical experimental procedures were followed for all three populations. Each population was grown in a natural crossing block at the Central Crops Research Station, Clayton, North Carolina in 1994. Each crossing block consisted of 1800 hills. Two seeds were planted in each hill. Hills were spaced 0.48 m within rows and 0.48 or 0.96 m between rows. Because insect pollination was possibly nonrandom, a grid system was used in sampling single male-sterile plants (Burton et al., 1990). Each intermating block was divided into 12 sub-blocks. Sixteen male-sterile plants with hybrid seed were harvested from each sub-block when their male-fertile siblings had reached harvest maturity.

Single MS plants were selected based on the density of their seeds. The two highest and two lowest seed density MS plants from each sub-block were selected. A modified liquid displacement method was used to determine seed density (Wessel-Beaver, et al. 1984). With this method, seeds with known weight are placed in a wire cage and immersed in a container of water on a tared balance. Volume of the seeds is the difference in weight of the container of water plus cage with and without the immersed seeds. Seed density is calculated as the product, seed weight × seed per volume. Because of low seed set on MS plants, the seeds of the 24 selected high density plants and 24 selected low density plants from each population were increased as families at the Agricultural Experiment Station, Isabela, Puerto Rico, in 1994-1995. At maturity, all of the MF plants in each row were bulk harvested. Because of a shortage of seeds in each population, only 15 out of 24 families in either high seed density group or low density group were kept for further testing. The experiment to evaluate the performance of these materials was conducted during the summer of 1995. The 15 lines of each density group were randomly divided into three sets for each population. One set of each density in each population was tested in a split-plot design at the Border Belt Tobacco Research Station, Whiteville, NC, Tidewater Research Station, Plymouth, NC, and Central Crops Research Station, Clayton, NC. Set was the main plot factor and seed density group was the subplot factor. There were two replications at each location. Three-row plots, with a row length of 5.8 m, were used. Row spacing was 0.97 m and 4.9 m of the center row was harvested. The cultivar Centennial was planted as the two outside border rows for each line in the three-row plot. The seeding rate was 19 to 23 seeds m^{-1}. Lines were planted on 24 May 1995, at Whiteville in soil mapped as Goldsboro fine sandy loam (fine, loamy, silecious, thermic, Acquic Paleudult); on 31 May 1995, at Plymouth in soil mapped as Portsmoth (fine-loamy/sandy or sandy skeletal, mixed, thermic, Typic Umbraquult); and on 4 July 1995, at Clayton in soil mapped as Wagram loamy sand (loamy, silecious, thermic, Arenic Paleudult) and Varina loamy sand (clayey, kaolinitic, thermic, Plinthic Paleudults).

At harvest maturity, the center row of each plot was trimmed to 4.9 m and the number of MS and MF plants was recorded. The frequency of MS plants was expected to be 0.25 on average, because of segregation among progeny of S_1 heterozygotes. The MS and MF plants were harvested and threshed separately. Seed yield was determined only on the MF plants from the center row. Seed density and seed weight per seed were measured for each line. A random 25-g sample from MF plants of each replicate at each location was analyzed with an infrared grain quality analyzer at the Northern Regional Research Center, Peoria, IL, to determine the concentrations of protein and oil. Density and weight of seeds from MS plants in each row were also measured.

Statistical Analysis

Male fertile seed density, seed weight, yield, and protein and oil concentrations were subjected to analysis of variance. Genotypic and phenotypic variances were estimated with variance components from analysis of variance (Johnson et al., 1955a). Analysis of variance of lines was performed by means of the general linear models procedure (PROC GLM) of SAS for each of the populations separately (SAS Institute Inc., 1985). Error variances across locations were homogeneous, so statistical analysis was done on the combined data from the three locations. Density group was considered to be fixed and all remaining factors were considered to be random. Both density group and set effects were non-significant in all populations (data not shown). Thus, these two factors were merged into one factor called set-group. Set-group included all the combinations of group and set (Table 1). Family mean squares that were significant at 0.05 level and location × family mean squares that were not significant were used to estimate genotypic and phenotypic variances and covariances for these traits. These estimates were used to calculate heritabilities, genotypic correlation coefficients, and expected genetic gains from selection for each trait in each population.

Table 1. Expected mean squares for the analysis of variance of two replications of 30 soybean families grown at three locations.

Source	df	Expected mean square‡
Locations	2	$\sigma_\delta^2 + n\sigma_\varphi^2 + r\sigma_{lg}^2 + rn\sigma_{ls}^2 + ns\sigma_{r(l)}^2 + rns\sigma_l^2$
Replications	3	$\sigma_\delta^2 + n\sigma_\varphi^2 + ns\sigma_{r(l)}^2$
Set-groups†	5	$\sigma_\delta^2 + n\sigma_\varphi^2 + rl\sigma_g^2 + rn\sigma_{ls}^2 + rlnK_s$
Location × set-group	10	$\sigma_\delta^2 + n\sigma_\varphi^2 + r\sigma_{lg}^2 + rn\sigma_{ls}^2$
Replications × set-group (location)	15	$\sigma_\delta^2 + n\sigma_\varphi^2$
Families (set)	24	$\sigma_\delta^2 + + r\sigma_{lg}^2 + rl\sigma_g^2$
Locations × families (set-group)	48	$\sigma_\delta^2 + r\sigma_{lg}^2$
Error	72	σ_δ^2

† The 15 lines in the high density group and 15 lines in the low density group were randomly assigned to sets of 5 lines each. Both the group effect and the set effect were nonsignificant. Thus, these two sources of variation were merged into one called set-group.
‡ r = number of replicates (r = 2), l = number of locations (l = 3), n = number of lines per set-group (n = 5), s = number of set-groups (s = 6).

Heritability on an entry-mean basis was calculated after Johnson et al. (1955a). Confidence intervals for heritability estimates were calculated after Knapp et al. (1985). Genetic variance estimates were derived from variance components using expected mean squares to determine appropriate formulae (Dudley and Moll, 1969). Heritability estimates (h^2) were used to predict selection progress. The change in population mean (ΔG) because of selection was calculated as follows:

$$\Delta G = k\sigma_p h^2$$

Where k = the standardized selection differential and σ_p is the phenotypic standard deviation. The correlated response of trait Y to selection on trait X ($\Delta G_{Y.\ X}$) was predicted by the following equation:

$$\Delta Gy.\ x = k\sigma_{py} h_y^2 h_x^2 r_A$$

where k is the standardized selection differential and σ_{py} is the phenotypic standard deviation for trait Y, heritabilities of the two traits are h_x^2 and h_y^2, and r_A is the genetic correlation between the two traits (Burton, 1987).

Genotypic correlation coefficients were calculated among MF seed density, MF seed weight, yield, and protein and oil concentrations (Johnson et al., 1955b). The standard errors of the genotypic correlations were computed following formulae presented by Mode and Robinson (1959). Two tailed t-tests were used to determine the statistical significance of genotypic correlation coefficients. Sums of squares and cross products were generated by the multivariate analysis of variance (MANOVA) in the GLM procedure of SAS (SAS Institute Inc., 1985).

A desired gains index was applied in each population (Baker, 1986). Male fertile seed density and MF seed weight were the restricted traits. The index weights for these two traits were calculated by solving the equation:

$$b = \text{Pi} \times \text{Gt} \times \text{inv}(G \times \text{Pi} \times \text{Gt}) \times K$$

where b = matrix of weights for seed density and seed weight, P = phenotypic variance-covariance matrix of the two traits, G = genotypic variance-covariance matrix of the two traits, Pi = the inverse of matrix P, Gt = the transpose of the matrix G, K = matrix of desired gains in a value of the two traits.

There were many options for determining matrix K, the desired gains. A screening procedure was applied to decide which matrix K to use for obtaining a matrix b that could lead to a significant increase in yield and protein concentration. We chose absolute values of correlated responses of seed density and seed weight to selection on protein as the desired gains. Matrix manipulations and calculations were performed using the interactive matrix language (PROC IML) of SAS (SAS Institute, Inc., 1985) for each of the populations. The index,

$$I = b_1 P_1 + b_2 P_2$$

where the b_1 and b_2 were weights to be given the corresponding characters (P_1 = seed density, P_2 = seed weight) used to compute the single index value, I. Heritability of I, genotypic correlations with other traits, and correlated responses expected from selection on I were calculated as with the other traits.

RESULTS AND DISCUSSION

Mass Selection

The initial evaluation in 1994 showed density of seeds from single MS plants was variable. The seed density ranges in Population II, III, and VII were 0.00541, 0.00244, and 0.00226 mg mm^{-3}, respectively. The difference between the average density of the 15 highest and 15 lowest selections was significant in all three populations (Table 2). Following one generation of seed increase of the selfed families MS and MF plants segre-

Table 2. Seed density and seed weight means of male-sterile soybean plants in high and low seed density groups grown at Clayton, NC, in 1994, and at Clayton, Whiteville, and Plymouth, NC, in 1995.

			1994		1995	
Population	Group	N†	Seed density	Seed weight	Seed density	Seed weight
			mg mm^{-3}	mg seed^{-1}	mg mm^3	mg seed^{-1}
II	High	15	0.01295	251	0.01194	187
	Low	15	0.01189	233	0.01192	190
	lsd.05		0.00051	46	0.00009	5
III	High	15	0.01253	234	0.01197	197
	Low	15	0.01196	228	0.01196	193
	lsd.05		0.00026	12	0.00009	6
VII	High	15	0.01273	249	0.01186	210
	Low	15	0.01226	265	0.01183	214
	lsd.05		0.00032	18	0.00052	6

† The 1994 means are averages of 15 single plants from one location.
The 1995 means are averages of 15 plot means, where each plot mean is the average of two replicates at three locations.

Table 3. Mean seed density, seed weight, seed yield, and concentrations of seed protein and oil of male-fertile plants in high and low seed density groups of three soybean populations grown at Clayton, Whiteville, and Plymouth, NC, in 1995.

Population	Group†	*N*‡	Seed density	Seed weight	Seed yield	Seed protein	Seed oil
			mg mm^{-1}	mg seed^{-1}	t ha^{-1}	g kg^{-1}	g kg^{-1}
II	High	15	0.01235	137	1.85	435	186
	Low	15	0.01233	139	1.90	434	187
III	High	15	0.01232	139	1.89	437	187
	Low	15	0.01230	140	1.90	439	185
VII	High	15	0.01227	147	1.96	414	205
	Low	15	0.01226	152	2.01	414	205

† The difference between the high and low group means were nonsignificant in all populations and traits.
‡ The means are averages of 15 plot means, where each plot mean is the average of two replicates at three locations.

gated in the plots. Results for each are reported here as derived MF or derived MS plants referring to progeny of the selfed families. If selection of increased and decreased seed densities of MS single plants were effective, then the means of seed density of either derived MS or derived MF plants from the high density group would be expected to be significantly higher than the mean density of the low density group. Although, average seed densities of derived MS and MF plants from the high density group were numerically higher than those from the low density group, in the following year the differences between the two were very small and nonsignificant (Tables 2, 3). Mean differences between the two groups in seed weight, seed yield, and concentrations of protein and oil were also non-significant. Thus, selection for seed density of single MS plants was not effective in altering any of the five traits. This was not due to lack of genetic variation. Variation among the 30 families in each population was significant for seed density, as well as seed weight, and concentrations of protein and oil (Table 4). Variation for seed yield was significant in Populations III and VII.

These results clearly show that seed density genotype cannot be determined by evaluating the single plant phenotype. Protein concentration is partly a function of N supply. Because the single plants were spaced between 0.48 m and 0.96 m apart, it is likely that there was variation among the plants in the soil N available for uptake. Evidence for this was found by Leffel and Hanson (1961) in a study of associations between generations of 45 diallel crosses. In that work, the simple correlation between seed protein of spaced F_2 plants and protein of F_3 bulks was found to be only 0.30. Furthermore, Weber and Horner (1957) showed that variation in protein concentration increased as plot size decreased. Thus, it is likely that plant-to-plant variation is too large, becaue of micro-environment, to distinguish genotypic differences in protein concentration adequately on an individual plant basis. Because seed density and protein concentration are correlated positively (Fehr and Weber, 1968; Smith and Weber, 1968; Li, 1996), the inability to determine the density genotype of single plants is probably due to plant to plant protein variation caused by variation in rhizosphere environment.

Family Selection

An alternative to mass selection for seed density would be S_1 progeny or selfed half-sib family selection (Burton and Carver, 1993). While such a system would require 2 yr instead of 1 yr to complete a cycle, selection for seed density might still have less negative impact on seed yield than selection for protein. If combined with seed weight in an index, simultaneous selection for both traits could be a relatively inexpensive way to increase protein and yield in a breeding population. Because location × family interactions were not significant for seed density and seed weight, it is likely that the two traits could be effectively evaluated in a small number of environments. While protein concentration is often affected by environmental influences, the relative rankings of genotypes usually are not (Kane et al., 1997). Brim and Burton (1979) successfully increased protein using single location evaluations of S_1 families and Tinius et al. (1991) increased seed size using single plant evaluation in a single location.

To evaluate the probable success of selection in this way, quantitative genetic parameters for the three populations were determined. Estimates of selfed half-sib

Table 4. Partial analysis of variance (mean squares) for seed related traits measured in two replications of 30 selfed half-sib families from soybean populations II, III, and VII grown at Clayton, Whiteville, and Plymouth, NC, in 1995.

Population	Source	df	Seed density	Seed weight	Seed yield	Seed protein	Seed oil	Seed index
II	Families(Set)	24	1.11×10^{-4}**	217.39**	0.0815	263**	100**	3.63**
	Loc × families(set)	47	0.50×10^{-4}	67.06	0.0551	91	34	1.72
	Error	71	0.44×10^{-4}	50.93	0.0511	72	32	1.94
III	Families(Set	24	1.74×10^{-4}**	308.48**	0.1080*	535**	165**	2.76**
	Loc × families(set)	47	0.57×10^{-4}	75.20	0.0605	114	29	0.69
	Error	71	0.67×10^{-4}	91.88	0.0771	82	26	0.93
VII	Families(Set)	24	0.77×10^{-4}*	293.64**	0.1186*	410**	144**	25.44**
	Loc × families(Set)	48	0.41×10^{-4}	55.85	0.0593	72	28*	5.62
	Error	72	0.45×10^{-4}	43.71	0.0873	57	16	4.51

* Indicates significance at $P = 0.05$.
** Indicates significance at $P = 0.01$.

Table 5. Heritability estimates (entry-mean basis), and 95% confidence interval for seed density, seed weight, seed yield, protein concentration and oil concentration in soybean population II, III, and VII.

Trait†	Heritability estimates		
	Population II‡	Population III‡	Population VII‡
Seed density	0.12 ≤ 0.55 ≤ 0.79	0.36 ≤ 0.67 ≤ 0.85	−0.04 ≤ 0.47 ≤ 0.75
Seed weight	0.40 ≤ 0.69 ≤ 0.86	0.52 ≤ 0.76 ≤ 0.89	0.63 ≤ 0.81 ≤ 0.91
Seed yield	−0.32 ≤ 0.32 ≤ 0.68	−0.09 ≤ 0.44 ≤ 0.74	0.03 ≤ 0.50 ≤ 0.76
Protein	0.33 ≤ 0.65 ≤ 0.84	0.58 ≤ 0.78 ≤ 0.90	0.66 ≤ 0.82 ≤ 0.92
Oil	0.34 ≤ 0.66 ≤ 0.84	0.66 ≤ 0.82 ≤ 0.92	0.66 ≤ 0.81 ≤ 0.92
Index	0.08 ≤ 0.52 ≤ 0.78	0.51 ≤ 0.75 ≤ 0.88	0.57 ≤ 0.78 ≤ 0.90

† Means of two replicates at three locations.
‡ Thirty half-sib families sampled at random from each population and selfed for one generation to increase seeds for testing.

family heritability (entry-mean basis) for seed density were lower in all three populations than heritabilities for seed weight, protein, and oil (Table 5). They were greater than heritabilities for seed yield in Populations II and III, but not Population VII. Confidence intervals for density heritability estimates were also smaller in Populations II and III than the intervals for yield heritability, but not in Population VII (Table 5). Genotypic correlations between seed density and protein were positive in all three populations, but significant only in Populations II and III. Correlations between seed density and seed weight and between seed density and yield were not significant. Seed weight and yield were correlated positively in Populations II and III. Thus in Population II at least, selection for higher seed density and seed weight should result in correlated increases in protein and yield. Protein and oil were negatively correlated in all three populations. But the correlations between oil and density while negative are not as large as those between oil and protein. Thus, selection for density might be a way to increase protein with less detriment to oil content in some populations.

Index Selection

Because seed density was positively correlated with protein content and seed weight was correlated positively with yield, we calculated a desired gains index for simultaneous selection of seed density and weight. In all three populations, the desired gain for seed density was the absolute value of the predicted correlated response of seed density to selection for seed protein concentration and the desired gain for seed weight was the value of the correlated response of seed weight to selection for seed protein. The expected gains matrix, K, and the index matrix, b, for Population II were $K' = (0.0135, 0.9321)$ and $b = (17.838, 0.0772)$; for Population III, $K' = (0.0175, 2.7028)$ and $b' = (8.971, 0.0679)$; and for Population VII, $K' = (0.0072, 1.2082)$ and $b = (11.65, 0.0283)$.

Heritabilities for the index were lowest (0.52) in Population II and highest in Populations III and VII, 0.75 and 0.78 respectively (Table 5). The index values had positive genotypic correlation coefficients with all traits in all populations except oil in Populations II and III (Table 6). Use of the index should result in positive increases in both protein and yield in all three populations (Table 7). Selection for either density or seed weight individually is expected to produce either less gain in protein than the index or less gain in yield. In Populations II and III selection for the index is expected to cause some decline in oil.

From these results, it appears that selection for a seed density-seed weight desired gains index would be an inexpensive way to improve protein concentrations and yielding ability in a breeding population. This could be a useful first step in improving a population derived from unadapted materials. Growing two or three replications of observation rows would provide data needed to select for density and seed size. Such observation rows could also allow selection against other undesirable

Table 6. Genotypic correlation coefficients with standard errors in parentheses for all pairs of seed related traits for populations II, III, and VII, derived from the analysis of 30 families from each soybean population grown in two replicates at three locations.

Pair of traits		Population		
		II	III	VII
Seed density and	Weight	−0.35 (0.30)	0.03 (0.28)	0.02 (0.33)
	Yield	0.21 (0.53)	−0.25 (0.38)	0.30 (0.41)
	Protein	0.90 (0.20)**	0.33 (0.25)	0.24 (0.30)
	Oil	−0.39 (0.29)*	−0.27 (0.26)	−0.49 (0.26)**
	Index	0.76 (0.14)**	0.69 (0.50)**	0.20 (0.32)
Seed weight and	Yield	0.81 (0.39)**	0.96 (0.26)**	0.19 (0.30)
	Protein	−0.17 (0.29)	0.36 (0.23)*	0.16 (0.24)
	Oil	0.46 (0.25)*	0.03 (0.25)	0.13 (0.24)
	Index	0.32 (0.30)	0.76 (0.12)**	0.99 (0.01)**
Seed yield and	Protein	0.05 (0.45)	−0.75 (0.30)**	0.12 (0.31)
	Oil	0.31 (0.44)	0.67 (0.29)**	−0.21 (0.31)
	Index	0.76 (0.56)**	0.53 (0.31)**	0.26 (0.30)
Protein and	Oil	−0.71 (0.15)**	−0.71 (0.12)**	−0.87 (0.06)**
	Index	0.78 (0.24)**	0.48 (0.22)**	0.21 (0.24)
Oil and	Index	−0.07 (0.34)	−0.16 (0.25)	0.03 (0.25)

* Indicates significance at $P = 0.05$.
** Indicates significance at $P = 0.01$.

Table 7. Expected direct and correlated responses to selection among selfed half-sib soybean families for seed density, seed weight, and seed density-weight index, presented as a percentage of population mean.

Population	Selection criteria	Response				
		Density	Seed weight	Seed yield	Seed protein	Seed oil
		%				
II	Density	0.26	−1.28	0.75	1.11	−0.70
	Seed weight	−0.10	4.10	3.23	−0.24	0.92
	Index	0.19	1.14	2.63	0.94	−0.12
III	Density	0.40	0.15	−1.31	0.70	−0.77
	Seed weight	0.01	5.31	5.36	0.81	0.09
	Index	0.29	4.01	2.94	1.08	−0.48
VII	Density	0.18	0.08	1.36	0.38	−0.94
	Seed weight	0.00	5.04	1.15	0.34	0.33
	Index	0.05	5.00	1.56	0.45	0.08

traits, such as disease susceptibility. If the observation rows were grown at two or more locations, better estimates of genotypic and phenotypic variances could be obtained for use in calculating the expected gains index. After one or more cycles of selection with this system, pure lines could be derived and evaluated by usual pedigree selection techniques. If the breeding population were segregating for male sterility, random intermating of selected selfed half-sib or S_1 families would be simple and inexpensive, making recurrent selection an option for as many cycles as are deemed useful. With such a population, introgression of new germplasm could be done by interplanting the new parental materials with the materials being intermated. The above is inexpensive because it requires relatively little time, land, and labor and no expensive instrumentation. Such a selection system could be easily incorporated into any soybean breeding program, whether large or small.

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